Exploring Heart Lymphatics in Local Drug Delivery

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ABSTRACT

Background: Local intramyocardial delivery (IMD) is under active clinical investigation for cell therapies to treat congestive heart failure, and gene therapies to induce revascularization of ischemic myocardium in coronary artery disease. Locally delivered agents can migrate away from the site of delivery through pathways that include lymphatics. Postdelivery redistribution can be observed using fluorescent tracers of different physical geometries. This approach provides a means to characterize these pathways and to delineate their importance in local cardiovascular drug delivery.

Methods and Results: The left ventricular wall of rats (N = 83) received injections of fluorescent microspheres with mean diameters ranging from 20 nm to 15,000 nm. Fluorescent microscopy was used to observe and image the patterns of migration from the epicardial surface. The animals were sacrificed after delivery. The microspheres with diameters smaller than 200 nm were widely distributed within the lymphatic network on the epicardial surface of the rat heart and through the ventricular wall at the injection site. Cardiac lymph nodes were identified with 20 nm and 100 nm deliveries, but could not be identified in any deliveries 200 nm or larger. The 15000 nm microspheres did not migrate.

Conclusions: Tortuous lymphatic pathways are apparent in the images of fluorescent sphere migration from the intramyocardial site of delivery. These images suggest a lymphatic role in the formation of native collaterals that may implicate potential advantages to IMD in therapeutic angiogenesis. Distribution postdelivery also suggests that IMD may provide a means to administer hydrophilic agents to the periadventitial zone of the arterial wall to limit restenosis. The lack of redistribution of the 15,000 nm microspheres supports the potential for cell therapies to remain localized over an extended time frame.

INTRODUCTION

INTEREST IN THE CARDIAC lymphatics has spanned hundreds of years. Investigations have been performed into their anatomical presentation and their involvement in pathological processes. Study of the cardiac lymphatics has often emphasized the delivery of an agent into the myocardial interstitium in order to identify, observe, and occasionally cannulate the cardiac lymphatic vessels. Here the importance of the lymphatics in local cardiovascular drug delivery will be considered.

Methods of intramyocardial infusion have recently been introduced to provide local delivery of therapeutic agents to the heart. It is believed that improved pharmacokinetics of these agents will ensue through local intramyocardial delivery, since local delivery to a tissue may provide high local concentrations

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while circulating blood concentrations remain low. Delivery of these agents to a region of the myocardium through open chest procedures for direct access has been explored in animal studies,[1–8] and has been used in clinical settings for therapeutic angiogenesis[9–11] and, more recently, for cell therapy.[12] Furthermore, percutaneous catheter systems for local delivery to a depth within the myocardium are currently being evaluated.[13–20]

The efficacy of any pharmacological therapy depends upon an accurate understanding of both the pharmacokinetics (time course of the body's exposure to drugs) and the pharmacodynamics (effect of a particular drug concentration on body tissue). Pharmacokinetics of local delivery must be understood in terms of the time course of the local drug distribution within the target tissue.[21] Parameters that affect local retention and postdelivery distribution include the physicochemical properties of both the agent and its carrier, the properties of the target tissue, and the means and methodology used for delivery. In addition to the requirement for an accurate assessment of local intramyocardial pharmacokinetics, there is also a need to understand the closely related transport pathways within the myocardium. These transport pathways are likely to be important both for signaling and for tissue remodeling. Locally delivered agents may migrate away from the site of delivery through lymphatics, veins, arteries, Thebesian vessels, and interstitial diffusion. A schematic of this complicated architecture is shown in Figure 1.

Early studies of lymphatic transport laid the groundwork of this field through observational studies of the intramyocardial delivery of India ink[22] and of contrast medium viewed radiographically.[1] To date, there have been no investigations of intracardiac delivery that systematically explore the variability of distribution as a function of agent physicochemical properties.

MATERIALS AND METHODS

This study protocol was approved by the Committee on Animal Research, University of California, San Francisco, and conducted in ac-

cordance with Federal guidelines. Rats were selected as an appropriate animal model for these investigations. Rats are frequently used in preclinical studies for intramyocardial therapeutic strategies. Their size enables the observation of changes in fluorescence in the heart over time. Rats (N = 83) scheduled for euthanasia were anesthetized with intraperitoneal Nembutal (40 mg/kg), intubated through a tracheotomy, and placed on a respirator at a rate of 100 strokes/minute, with a stroke volume of 1 cc. A median sternotomy was performed, and the left ventricular wall was presented by grasping the right ventricular outflow tract and lifting the heart with a forceps.

Fluorescent microspheres (Molecular Probes, Eugene, OR) were utilized for injection into the myocardium. The sizes of the microspheres were selected to approximate what is commonly employed for cell therapy and appropriate controlled release formulations (15 μm), adenovirus formulations (200 nm and 100 nm), and adeno-
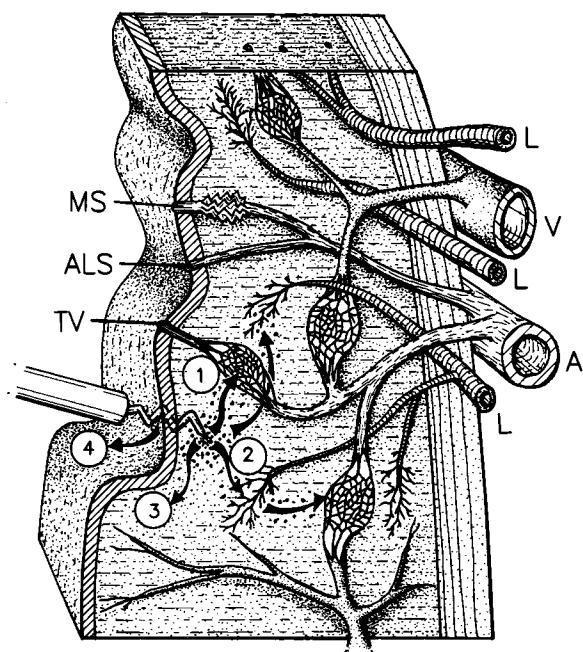
FIG. 1. Cross-sectional schematic of the left ventricular free wall showing an endocardial infusion system with a hollow helical needle delivering a medium to the myocardium. Arrows show various routes of egress from the delivery site including uptake into lymphatics, veins, and arteries, as well as interstitial diffusion and thebesian vein drainage.

associated virus formulations (20 nm). The microspheres were injected in volumes of 50 and 100 μL into the left ventricular wall with a 30-gauge needle attached to a tuberculin syringe. Injections were performed under a dissecting microscope. Care was taken to inject at an angle of approximately 45°, oblique to the ventricular surface. Fluorescent microscopy was used to observe and image the patterns of migration from the epicardial surface. The animals were sacrificed at 1, 10, and 100 minutes after delivery. The hearts were examined in cross section.

RESULTS

Individual cardiac specimens were eliminated from further scrutiny if technical limitations precluded adequate analysis. These included surgical imprecision during the delivery process (N = 6), back leak of the injectate to the epicardial surface (N = 4), and loss of the animal due to anesthesia and surgical trauma (N = 1).

Seventy-two deliveries were believed to have been well performed and suitable for further study. Care was taken to carefully observe the unique characteristics of each delivery and to map out the efferent lymphatics of the rat heart, as has been previously described.[23]

In all cases, the subepicardial lymphatic vessels were seen to be connected in a grid. Connections between vessels occurred at nearly right angles. This network extended over both ventricles. Migration was observed among the atrial structures and up to a lymph node located on the right dorsal region of the mediastinum.

Figures 2, 3, and 4 show the distribution within the rat myocardium of fluorescent microspheres of varying diameters. The 20 nm and 100 nm microsphere deliveries appeared to be similar in all cases. Rapid migration within the epicardial lymphatic plexus was immediately apparent, resembling a hydraulic connection between this network and the site of interstitial delivery. Cross-sectional distribution of 20 nm and 100 nm spheres was observed through the full thickness of the ventricular wall in most cases; however, the area was relatively limited to the site of delivery, with more extensive migration observed primarily in the subepicardial lymphatic plexus and, occasionally, within a subendocardial collecting vessel whose identity could not be confirmed as a lymphatic. Once delivery was completed, the pattern of microsphere distribution in the subepicardial lymphatic network did not appear to change significantly and no consistent differences were observed at 1, 10, and 100 minutes. Twenty nm spheres were most easily traced through the lymphatics to thereby identify a distinct lymph node above the right side of the heart. In one instance, the lymph node was isolated after 100 nm sphere delivery.
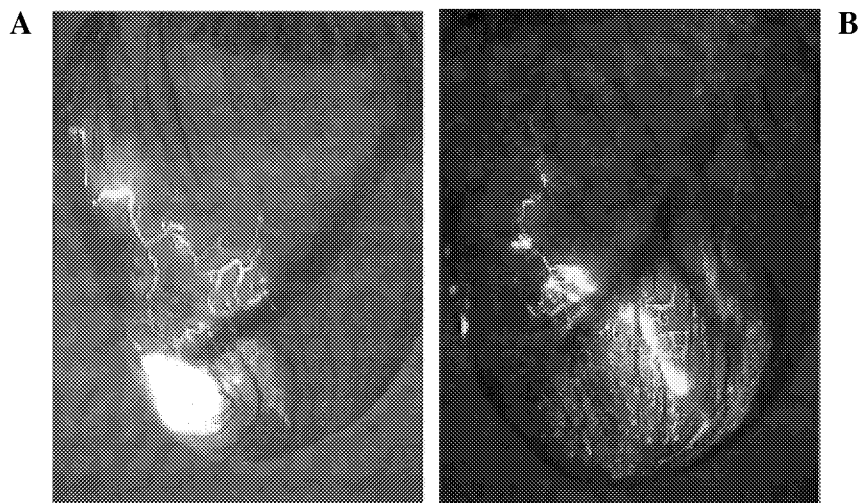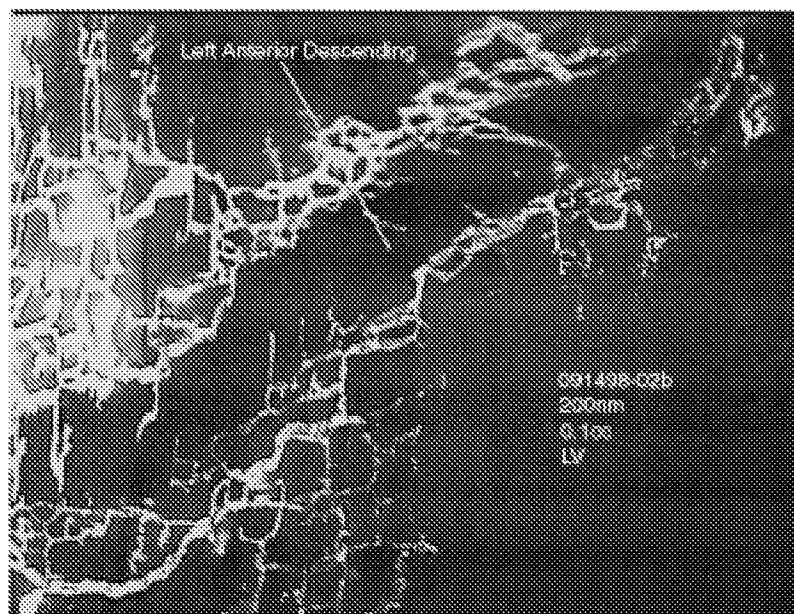

FIG. 2. Epicardial presentation of 20 nm microspheres delivered to the rat heart in vivo showing the difference between a 50 μL volume after 100 minutes (**A**) and a 25 μL volume after 1 min (**B**).

A   B
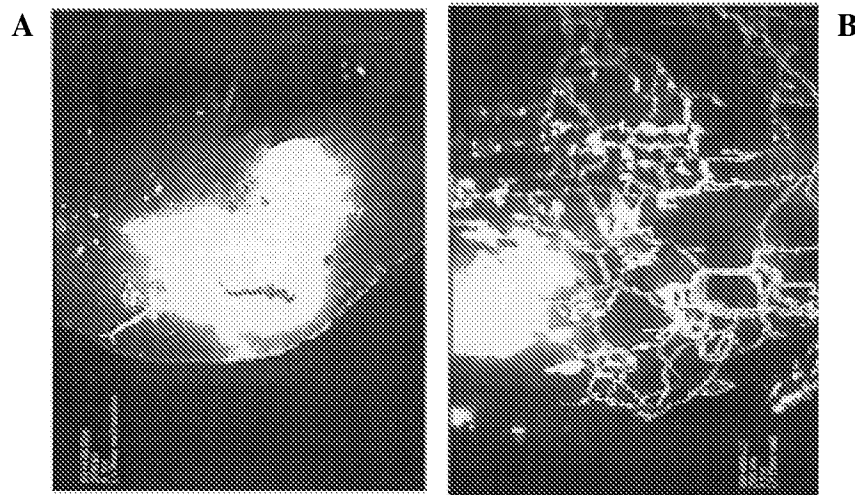
FIG. 3. Cross-sectional (**A**) and epicardial (**B**) presentation of 200 nm microspheres delivered in a volume of 100 μL to the rat heart *in vivo* after 10 minutes.

The larger, 100 μL, delivery volume resulted in a larger volume of distribution, both in the cross-sectional and subepicardial views. Although twice the volume was delivered, these distributions increased only by an additional 50% in area, within each view. Quantification was difficult because of inconsistencies in the location at which the heart was sectioned and in the epicardial presentation.

Deliveries of 200 nm microspheres differed markedly from the smaller spheres, because the larger spheres migrated preferentially up the left anterior descending artery, with less diffuse distribution. There was significant migration towards the apex of the heart, with a distinct starburst pattern. In cross section, the spheres were seen both in a localized and in a wider distribution, the latter through the entire

FIG. 4. Epicardial presentation of 200 nm microspheres delivered in a volume of 100 μL to the rat heart *in vivo* after 10 min, demonstrating migration of this sphere up the left anterior descending coronary artery.

cross section of the heart. Neither subepicardial presentation nor cross-sectional changes were observed to be consistently different at 1, 10, or 100 minutes. As expected, larger volumes spread over larger areas in the subepicardial distribution, but not in cross section. In general, spheres that had diameters of 20, 100, and 200 μm were observed to distribute substantially within the lymphatic network that was apparent on the epicardial surface of the rat heart. They also distributed throughout the ventricular wall at the injection site. However, lymph nodes could not be identified in any 200 nm deliveries.

Deliveries of 15,000 nm microspheres remained far more localized than those of the smaller spheres. Although wide distributions of the spheres were seen under fluorescence microscopy, these spheres were less continuously distributed and less clearly defined than the lymphatic pathways in the subepicardial plexuses. In sectioning, transmural distributions were also reduced in comparison to all other sphere sizes. Over 10 to 100 minutes there appeared to be significant migrational changes, or increased epicardial distribution. No lymph nodes could be identified in any 15,000 nm microsphere deliveries.

In two of the formalin-fixed hearts, an attempt was made to identify the structures more conclusively as lymphatic, through histochemical means. PAL-E/CD31, anti -VEGFR-3, and LYVE-1 antibody stains were attempted, as has previously been described.[24] Specific staining was not observed, most likely as a consequence of prolonged storage of the tissues in formalin (for periods exceeding 1 year). Nevertheless, the morphology observed under fluorescence microscopy and the occasional, clearly identi-

fied lymph nodes are highly suggestive of the lymphatic identity of the vessels.

DISCUSSION

Delineation of the transport mechanisms within the myocardium is important to understand the potential benefits and advantages of local intramyocardial drug delivery. There is potential for such drug delivery: intramyocardial therapeutics might overcome problematic systemic effects, as well as provide an avenue for three-dimensional vascular remodeling.

The probes selected for delivery in this study are appropriately sized to mimic the therapeutic agents that have been proposed for delivery to a specified depth within the myocardium: the adeno-associated virus is approximately 20 nm in diameter, the adenovirus is approximately 120 nm in diameter, and cellular and controlled release formulations are approximately 15000 nm in diameter. Physical size was deemed the most important of the physicochemical properties to evaluate among available formulations, although there are many others that could have a significant effect upon the outcome, as shown in Table 1.

The morphology of the 20 nm and 200 nm microspheres demonstrates tortuous pathways emanating from the site of delivery across the heart. This pattern is similar to what is seen in the protective native collateral vessels found in some patients presented with coronary artery disease. Although these corkscrew-shaped vessels are readily recognizable in the heart and the periphery, no rationale for this collateral vessel morphology has been suggested. Hypothetically, when one considers the release of endoge-

TABLE 1. PARAMETERS RELATED TO INTRAMYOCARDIAL THERAPEUTIC FORMULATION

Parameter	Variations
Agent type	Gene therapy (viral vector, naked DNA, plasmid, formulated plasmid, cellular), protein, controlled release formulation, cellular material
Physicochemical properties	Size, MW, charge, lipophilicity, globular versus linear, binding affinities
Carrier properties	Viscosity, pH, temperature, osmolality, presence of vasoactive agents

nous growth factors from hypoxic and injured tissues, it is reasonable to suspect that these agents would enter the lymphatics. In this scenario, the lymphatic pathway that carries the most clearly defined directional concentration gradient may be important in the tissue remodeling processes that respond to this concentration gradient. Furthermore, in the case of collateral formation, one may hypothesize that the lymphatic vessel could serve as the framework upon which such remodeled vessels are built. This hypothesis might be tested using the PAL-E/CD31 anti-VEGFR-3 and LYVE-1 antibody stains on cadaver hearts.

The similarity in subepicardial transport between 20 nm and 100 nm spheres, with differences observed at 200 nm, suggests that there are a large number of lymphatic vessels in the subepicardial surface that are below 200 nm in diameter. Furthermore, the observations in this study suggest that the vessels that lie along the left anterior descending artery are larger vessels. Either they exceed 200 nm in diameter or they are the first vessels to expand in size when no other routes of egress are presented.

The observation of lymphatic vessels filled with particulates surrounding the coronary arteries supports the hypothesis that periadventicial delivery to the arterial wall may be possible via an intramyocardial delivery approach. Lymphatic redistribution after intramyocardial delivery presents a new paradigm for the targeting of therapeutic agents locally to the coronary artery. Intramyocardial delivery of a small molecule hydrophilic agent within a controlled release formulation that is between 200 and 50,000 nm may entail optimal pharmacokinetics for the prevention of restenosis after percutaneous coronary intervention.

The appearance of spheres with 15,000 nm diameters at destinations remote from the site of delivery suggests that they can be carried through lymphatic vessels when supported by a significant pressure gradient. However, the lack of continuous transport pathways with the 15,000 nm diameter microspheres suggests that, in the rat heart, that there are few lymphatic vessels that readily accommodate particles of this size.

It is recognized that this observational study presents no more than a starting point for an attempt to understand the important transport pathways that subserve intramyocardial pharmacokinetics. Also, care should be used in extrapolating these observations to other species. This work can be furthered by exploring intramyocardial transport using radioactive and fluorescent probes whose migration after delivery may be measured.

ACKNOWLEDGMENTS

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Panel Discussion

FGF in cardiac lymph during ischemia

Dr. David Zawieja (Texas A&M University): It is interesting to hear you talk about delivery into the myocardium. There have been some recent studies in which the investigators attempted to occlude the coronary arteries, cannulated the lymph channels, collected the lymph, and measured FGF content. The study was carried out over the course of days. They were actually able to demonstrate substantial augmentation of FGF in the lymph exiting the cardiac tissues.

Mr. Altman: We have done work with cells, genes, radiolabeled albumin, and radiolabeled FGF. In one study, we cannulated the ascending aorta and the coronary sinus to track the concentrations of radiolabelled albumin in the blood immediately following delivery. We've observed a more rapid rise in concentration immediately after delivery from the coronary sinus, but not uniformly.